## REMARKS

Claims 1 and 17 have been amended to state that the incorporation or non-incorporation of each nucleotide is determined by "bioluminometrically detecting the release of pyrophosphate." Support for this amendment may be found in the substitute specification, for example at page 35, lines 9-10. In view of the amendment of Claims 1 and 17, Claims 4, 20, 24 and 25 have been cancelled without prejudice, and Claims 5 and 21 have been amended to correct dependencies.

In the Office Action mailed December 10, 2004, Claims 1, 7, and 17 have been rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 6,566,101 to Shuber et al. ("Shuber et al."). The Examiner has alleged that Shuber et al. teach a method for selective nucleic acid sequence detection in single primer extension reactions of high sensitivity, and that the method includes every limitation of Claims 1, 7 and 17.

Applicant respectfully submits that Shuber et al. fail to teach every element of the methods of Claims 1, 7 and 17 and therefore fail to anticipate the present invention. Shuber et al. teach a method comprising a single base primer extension. Shuber et al. at Col. 8, l. 40-42. The single base primer extension is performed by extending the 3' end of an annealed primer with a chain terminating nucleotide. Shuber et al. at Col. 8, l. 63 – Col. 9, line 1.

Shuber et al. do not teach at least the steps of performing a primer extension reaction by sequentially adding non-chain terminating nucleotides, or determining incorporation or non-incorporation as each nucleotide is added. Further, Shuber et al. do not teach the step of determining incorporation by bioluminetrically detecting the release of pyrophosphate. In addition, Shuber et al. do not teach the step of determining the frequency of an allele from a pattern of nucleotide incorporation. Rather, the reference teaches only a comparison between a number of a target nucleic acid in a patient sample and the number expected in a healthy patient to determine the presence or absence, not the frequency, of a mutation. Accordingly, Shuber et al. fail to anticipate the method of Claims 1, 7 and 17, and withdrawal of the rejection under 35 U.S.C. § 102(e) is respectfully requested.

Claims 4-7 and 20-25 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by WO98/28440 to Nyren ("Nyren-2") in view of Shuber et al. The Examiner has alleged that Nyren-2 teaches a method of sequencing DNA based upon detection of the release of pyrophosphate, and that it would have been obvious to modify the method of Nyren-2 with the pooling primer extension method of Shuber et al.

Applicant respectfully submits that Shuber et al. do not teach a step of pooling of nucleic acids in a population in order to determine the frequency of an allele in a population of nucleic acids. Even if, arguendo, Shuber et al. taught such a step, one would not have been motivated to combine Shuber et al. with Nyren-2, because one would not have expected that the method of Nyren-2 would be sensitive and accurate enough to accurately quantify allele proportions in a pooled sample.

Shuber et al. do not teach a method for determining the frequency of an allele in a population. Rather, Shuber et al. teach a method for detecting the presence of a single nucleotide, e.g. a mutation, in a patient sample. Shuber et al. at Col. 4, l. 48-55. Shuber et al. teach that the method is useful to detect and identify mutations associated with a disease. Shuber et al. at Col. 8, l. 54-62. The method does not require a determination of the exact amount of mutation present in a sample, but rather only a determination of a statistically significant difference from the absence of the mutation in a healthy individual, i.e., a statistically significant difference from zero. Shuber et al. at Col. 14, l. 59-62. In contrast, the claimed method of determining the frequency of an allele in a population provides a pattern of nucleotide incorporation that permits the quantitative analysis necessary for allele frequency determination. See substitute specification at p. 54, l. 2-24. Thus Shuber et al. fail to teach at least the step of determining the frequency of an allele from a pattern of nucleotide incorporation, as presently claimed. Rather, the reference teaches only a comparison between a number of a target nucleic acid in a patient sample and the number expected to be in a healthy patient in order to determine the presence or absence, not frequency, of a mutation. Thus even if one had been motivated to combine Shuber et al. with Nyren-2, the combination would not achieve the present invention.

The Examiner has alleged that Shuber et al. teach, at Col. 14, lines 60-68, that samples may be pooled to determine the number of a nucleic acid in a sample. However, with respect to

pooling, Shuber et al. teach only that samples from healthy individuals may be pooled to determine the number of nucleic acid in a healthy patient, i.e. a reference or control non-mutated sample against which a target sample is compared in order to diagnose a disease in an individual patient. Shuber et al. do not teach or suggest pooling of target samples, i.e. the samples in which a polymorphism or mutation is to be detected, as claimed in accordance with the present invention. Accordingly, there would have been no motivation to combine pooling as taught by Shuber et al. with the teachings of Nyren-2, and in any event the combination would not achieve the present invention.

The Examiner has alleged that Shuber et al. teach that pooled samples are useful to screen large number of individuals. However, rather than pooling of samples, Shuber et al. describe their invention as one that "accommodates the simultaneous screening of large numbers of nucleic acids from different patients with a large number of first probes that are complementary to mutations in more than one potential disease-causing gene." Shuber et al. at Col. 28, l. 23-28, emphasis added. In other words, multiple different primers are used for simultaneous analysis of multiple diseases in a patient. This simultaneous analysis is unrelated to pooling of samples.

The Examiner has further alleged that Shuber et al. teach that the primer extension to identify a single nucleotide polymorphic variant may be performed on combined samples. Shuber et al. at Col. 33, l. 10-15. Claims 19 and 20 of Shuber et al. refer to a method of identifying a single nucleotide polymorphic variant. There is no teaching or suggestion that combining samples would allow anything more than the identification, i.e. the presence, of a single nucleotide polymorphic variant. There is no disclosure in the specification of Shuber et al. that corresponds to Claim 20, and no teaching or suggestion of any use or benefit in combining samples, and hence no motivation to combine this isolated sentence of Shuber et al. with any other reference.

As the Examiner has acknowledged, Nyren-2 does not teach pooling of nucleic acids, or determination of the frequency of an allele in a population. Applicant submits that even if Shuber et al. taught frequency determination, which it does not, one would not have been motivated to modify the method of Nyren-2 to determine the frequency of an allele in a

population, because one would not have expected that the method of Nyren-2 would be sufficiently quantitative for such an application.

In support thereof, submitted herewith is the Declaration under Rule 132 of Pal Nyren, the sole inventor of Nyren-2. Dr. Nyren is an expert in the Pyrosequencing™ method disclosed by Nyren-2, and a stockholder of Biotage AB, the assignee of the present application.

In Dr. Nyren's expert opinion, Nyren-2 is concerned only with methods of determining base sequence information, and does not suggest that the method may be useful for purposes other than sequencing and related applications such as detection of single base changes. Declaration of Pal Nyren ("Decl.") at ¶ 5. In Dr. Nyren's opinion, one of skill in the art would not have been motivated to use the method of Nyren-2 for any further purpose, particularly for determining allele frequencing in a pooled population. Decl. at ¶ 5.

One would not have been motivated to modify the method of Nyren-2 for allele frequency determinations on pooled samples because such applications require much higher levels of accuracy, sensitivity and specificity than sample sequencing or base detection. Decl. at ¶ 6. As explained by Dr. Nyren, a highly quantitative method is not required to determine whether a sample from an individual is heterozygous or homozygous. In fact, the method need not be quantitative at all. This is because even with a background signal of, for example 20%, it is easy to distinguish if a single SNP is a homozygote (i.e. 100% A or 100% G in Dr. Nyren's example) or a heterozygotes (i.e. 50% A plus 50% G.) However, in a pooled sample of homozygotes and heterozygotes, much higher levels of accuracy are required. For example, in a pooled sample from 100 individuals of which 50% are homozygotes having only allele A and 50% are heterozygotes (resulting in 75% allele A and 25% allele G), a non-exact quantitative method is insufficient. For example, a non-exact method might indicate that 20% have allele G and 80% have allele A even if there is no allele G in the sample, i.e. in this example a 20% background signal cannot be tolerated. Decl. at ¶ 7.

According to Dr. Nyren, at the time of the present invention it was known that methods of sequencing such as traditional Sanger sequencing were not sufficiently accurate and quantitative to perform allele frequency determinations with pooled samples. Decl. at ¶ 8. Given the inaccuracy and unsuitability of other well known sequencing methods, it was not

expected that the method of Nyren-2 would be sufficiently quantitative or accurate. Rather, it was surprising that the method of Nyren-2 provided sufficiently quantitative data that correlated well enough to allele frequencies to enable allele discrimination and quantification. Decl. at ¶ 9.

For all of the foregoing reasons, Applicant respectfully submits that there was neither motivation to combine the references nor an expectation of success in so doing. Further, even if the references are combined, the present invention is not achieved because neither Shuber et al. nor Nyren-2 teaches the pooling of samples or the step of determining the frequency of an allele in a pooled population. Withdrawal of the rejection of Claims 4-7 and 20-25 is respectfully requested.

Claims 8-11, 14 and 15 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Shuber et al. in view of Breen et al. (2000) Biotechniques 28:464-470. Claims 8-15 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Shuber et al. in view of Germer et al. (2000) Genome Research 10:258-266. The Examiner has alleged that it would have been obvious to modify the method of Shuber et al. with the teachings of Breen et al. or Germer et al. that concentrations of samples should be ensured.

For all the reasons discussed hereinabove, Shuber et al. fail to teach or suggest at least: the step of performing a primer extension reaction by sequentially adding non-chain terminating nucleotides; the step of determining incorporation or non-incorporation as each nucleotide is added; the step of determining incorporation by bioluminometrically detecting the release of pyrophosphate; and the step of determining the frequency of an allele from a pattern of nucleotide incorporation. Neither Breen et al. nor Germer et al. remedy the deficiencies of the primary reference. Accordingly, even if one were motivated to combine the references, the combinations fail to achieve the present invention. Withdrawal of the rejections of Claims 8-15 under 35 U.S.C. § 103(a) is respectfully requested.

In view of the foregoing comments and amendments, it is respectfully submitted that the present application is in condition for allowance. Favorable reconsideration and allowance of all pending claims is earnestly solicited.

Respectfully submitted,

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